

**U.S.S.N. 09/601,645**  
**DAHM *et al.***  
**AMENDMENT**

**IN THE CLAIMS:**

A listing of the claims, in accordance with the revision of 37 CFR §1.121, is provided. The listing of claims replaces all prior such listings of claims. Claim 1 is amended herein. Claims 70, 71, 73-78 and 86 are cancelled herein.

1. (Currently Amended) A method for the quantification of tumor cells in a body fluid, comprising:

(a) concentrating tumor cells in a sample of a body fluid by covering a cell separation medium with a density in the range of from ~~1.060-1.067~~1.060-1.065 g/ml with a layer of the body fluid, centrifuging the cell separation medium covered with the body fluid and collecting the tumor cells at the interface of the cell separation medium and the supernatant body fluid;

(b) specifically amplifying, from the tumor cells, mRNA coding for the catalytic subunit of telomerase;

(c) quantitatively determining the amount of amplified nucleic acid; and

(d) correlating the amount of amplified nucleic acid with the number of tumor cells in the body fluid.

2. (Previously Presented) The method of Claim 1, further comprising prior to amplification, preparing cDNA from the mRNA contained in the sample.

3. (Previously Presented) The method of Claim 2, wherein, prior to preparing cDNA, the sample is treated with a DNAase.

4. (Previously Presented) The method of Claim 1, wherein the sample is gel purified.

5. (Previously Presented) The method of Claim 1, wherein for quantitative determination of the telomerase-coding nucleic acid, the amplification products are labeled during amplification and the amplification kinetics are measured continuously, including during the amplification process.

6. (Previously Presented) The method of Claim 5, wherein a probe that is specific for the amplification products, and that emits a characteristic signal proportional to the products amplified per synthesis cycle, is present during amplification.

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7. (Previously Presented) The method of Claim 1, wherein for quantitative determination of the telomerase-encoding nucleic acid, at least one standard nucleic acid molecule is coamplified and added in different concentrations to the sample.

8. (Previously Presented) The method of Claim 1, wherein the amplification product is quantified either directly or via a label.

9. (Previously Presented) The method of claim 1, wherein the amplification product is detected via hybridization with a labeled oligonucleotide.

10. (Previously Presented) The method of Claim 7, wherein quantification of the telomerase-encoding nucleic acid is effected by comparing the amount of coamplified nucleic acid or nucleic acids with the amount of telomerase-encoding nucleic acid.

11. (Previously Presented) The method of claim 1, wherein the sample is peripheral blood.

12. (Previously Presented) The method of Claim 1, wherein as a negative control water is employed in place of the body fluid.

13. (Previously Presented) The method of Claim 1, wherein one or both of the following oligonucleotide primers are used for the amplification:

5' CTACCGGAAG AGTGTCTGGA GCAAGTTGGA AAGC 3' SEQ ID No. 1, designated TRT1; and

5' GGCATACCGA CGCACGCAGT ACGTGTTCTG 3' SEQ ID No.2, designated TRT2,

wherein each of hTRT1 and hTRT2 optionally further comprises a promoter sequence for an RNA polymerase.

14. (Previously Presented) The method of Claim 1, wherein amplification is effected with a DNA polymerase or an RNA polymerase

15. (Previously Presented) The method of claim 14, wherein, if amplification is effected with a DNA polymerase, the amplification reaction is a polymerase chain reaction (PCR) and, if amplification is effected with an RNA

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polymerase, the reaction is an isothermal nucleic acid sequence-based amplification (NASBA) reaction.

16. (Previously Presented) The method of claim 1, wherein the sample is blood that is depleted in stem cells and/or activated immune cells.

17. (Previously Presented) The method of Claim 1, wherein the sample is blood, and the tumor cells from the blood sample are concentrated.

18. (Previously Presented) The method of Claim 1, wherein the cells contained in the sample are cultivated under conditions that are unfavorable for telomerase-positive nontumor cells but favorable for the tumor cells present.

19. (Previously Presented) The method of Claim 18, wherein the duration of the cultivation is such that nontumor cells die and tumor cells survive.

Claims 20 and 21 (Cancelled)

22. (Previously Presented) The method of Claim 1, wherein the centrifugation is carried out at about 1000 x g for about 30 minutes.

23. (Previously Presented) The method of Claim 1, wherein the cell separation medium used is Percoll or Ficoll.

24. (Previously Presented) The method of Claim 1, wherein the body fluid is blood and prior to applying the body fluid sample to the cell separation medium, the body fluid is mixed with one or more substances that prevent aggregation of platelets to tumor cells, and/or prior to applying the body fluid sample to the cell separation medium, the body fluid is freed of substances that promote aggregation of platelets to tumor cells.

25. (Cancelled)

26. (Previously Presented) The method of Claim 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent.

27. (Previously Presented) The method of Claim 11, wherein the peripheral blood is venous or arterial blood.

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28. (Previously Presented) The method of Claim 1, wherein the body fluid is selected from the group consisting of lymph, urine, exudates, transudates, spinal fluid, seminal fluid, saliva, fluids from natural or unnatural body cavities, bone marrow and dispersed body tissue.

29. (Previously Presented) The method of Claim 1, wherein after centrifugation and before collecting the tumor-cell-enriched interface, the centrifugation vessel is removed and cooled to prevent mixing of the cells in the different layers.

30. (Previously Presented) The method of Claim 1, wherein the centrifugation is carried out in a vessel that is divided by a porous barrier, a filter or a sieve into an upper and a lower compartment and the body fluid is introduced into the upper compartment.

31. (Previously Presented) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a thickness of 1-10 mm.

32. (Previously Presented) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a pore size of 20-100  $\mu\text{m}$ .

33. (Previously Presented) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve is fabricated from a hydrophobic material or coated with a hydrophobic material.

34. (Previously Presented) The method of Claim 1, wherein a dye is added to color the cell separation medium, whereby the color of the cell separation medium is distinguishable from that of the supernatant body fluid.

35. (Previously Presented) The method of Claim 1, wherein: the sample is blood;

the method is performed on venous blood sample and on an arterial blood sample; and the results from each are compared with one another.

36. (Previously Presented) The method of Claim 1, wherein: the sample is blood;

the method is performed on a blood sample from the finger pad and, on a venous or arterial blood sample; and

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the results from each are compared with one another.

37. (Previously Presented) The method of Claim 1, wherein the tumor cells are derived from metastases of malignant tumors.

38. (Previously Presented) The method of Claim 1, wherein the tumor cells are selected from cells of metastasizing tumors and/or neoplasms, wherein the cells are derived from tumors and cells selected from the group consisting of a T-cell lymphoblastoma, T-cell leukemia cells, chronic myeloid leukemia cells, acute lymphatic leukemia cells, chronic lymphatic leukemia cells, teratocarcinoma, melanoma, carcinoma of the lung, large intestine cancer, breast cancer, hepatocellular carcinoma, kidney tumor, adrenal tumor, prostate carcinoma, neuroblastoma, brain tumor, rhabdomyosarcoma, leiomyosarcoma and lymphoma cells.

Claims 39-51 (Cancelled)

52. (Original) The method of Claim 4, wherein purification is effected by ion exchange chromatography.

53. (Original) The method of Claim 52, wherein ion exchange resin is a silica gel.

54. (Original) The method of Claim 7, wherein three standard nucleic acids are coamplified and are added in different concentrations to the sample.

55. (Original) The method of Claim 8, wherein:  
the amplification product is quantified via a label; and  
the label is selected from a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

56. (Original) The method of Claim 9, wherein the label is a radioactive label, a biotin label, a fluorescent label or an electrochemoluminescent label.

57. (Original) The method of Claim 11, wherein, as a positive control in the sample, a nucleic acid that occurs in peripheral blood is specifically amplified and detected.

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58. (Original) The method of Claim 57, wherein the nucleic acid is mRNA that encodes a protein selected from among  $\beta$ -globin, glyceraldehyde-phosphate dehydrogenase,  $\beta$ -actin or a T-cell receptor.

59. (Original) The method of Claim 3, wherein as a negative control no reverse transcription reaction is carried out before the amplification reaction with the sample to be investigated and/or water is employed in place of the body fluid.

60. (Original) The method of Claim 16, wherein depletion is effected by immunoabsorption.

61. (Previously Presented) The method of Claim 17, wherein concentration is effected by immunoabsorption.

62. (Cancelled)

63. (Previously Presented) The method of Claim 1, wherein the density is about 1.065 g/ml.

64. (Previously Presented) The method of Claim 11, wherein the peripheral blood is drawn in an anticoagulant substance and, prior to covering the cell separation medium, diluted with a diluent at a ratio of about 1:1.

65. (Original) The method of Claim 31, wherein at least one of the porous barrier, the filter or the sieve has a thickness of about 5 mm.

66. (Original) The method of Claim 30, wherein at least one of the porous barrier, the filter or the sieve has a pore size of 20-30  $\mu$ m.

67. (Original) The method of Claim 1, wherein the tumor cells are derived from micrometastases of malignant tumors.

68. (Cancelled)

69. (Previously Presented) The method of Claim 1, wherein the tumor cells are separated from telomerase-positive non tumor cells.

Claims 70-86 (Cancelled)